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ORIGINAL ARTICLE

Diterpenoids and Limonoids from the Leaves and Twigs of *Swietenia mahagoni*

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Abstract Three new compounds, including two diterpenoids, nemoralisins H and I (**1** and **2**), and a limonoid, 2-methoxy khayseneganin E (**3**), along with four known constituents (**4**–**7**), were isolated from the leaves and twigs of *Swietenia mahagoni*. Their chemical structures were elucidated by means of spectroscopic analysis. The cytotoxities of these isolated constituents were assayed.

Keywords Meliaceae · *Swietenia mahagoni* · Diterpenoids · Limonoids

1 Introduction

Swietenia mahagoni, which is an economically important timber tree, has been used as a folk medicine for the treatment of hypertension, diabetes, and malaria [1, 2]. Chemical investigations conducted previously on *S. mahagoni* had led to the isolation of various B,D-*seco* limonoids such as mexicanolides, phragmalins, andirobins, gedunins, and rearranged phragmalins [3–7]. Modern pharmacological studies demonstrated that limonoids from *Swietenia* displayed insecticide, antitumor, antibacterial, antidiabetic, and antidyslipidemic activities [8–12].

With the aim of searching for structurally unique and bioactive chemical constituents, we isolated the leaves and twigs of *S. mahagoni* and gained two new diterpenoids (**1** and **2**), one new limonoid (**3**) and four known compounds (**4**–**7**) (Fig. 1). In this paper, we reported the isolation and structural elucidation of new compounds, as well as cytotoxicities of all the compounds against five human cancer cell lines.

2 Results and Discussion

The air-dried powder of leaves and twigs of *S. mahagoni* was extracted with 70 % aqueous acetone at room temperature three times to give the residue, which was then partitioned between EtOAc and water to get the EtOAc-soluble fraction. Then, three new constituents together with four known compounds were acquired by a series of chromatographic methods. Herein, we described the isolation and structural elucidation of these new compounds.

Compound **1**, colorless oil, was assigned a molecular formula $C_{20}H_{28}O_5$ according to its positive HREIMS peak at *m/z* 348.1932 $[M]^+$ (calcd for 348.1937), suggesting seven degrees of unsaturation. The IR spectrum revealed characteristic bands corresponding to hydroxyl (3,434 cm^{-1}), α,β-unsaturated-δ-lactone (1,761 cm^{-1}) and double-bond (1,639 cm^{-1}) groups. The ^1H NMR spectrum of **1** (Table 1)

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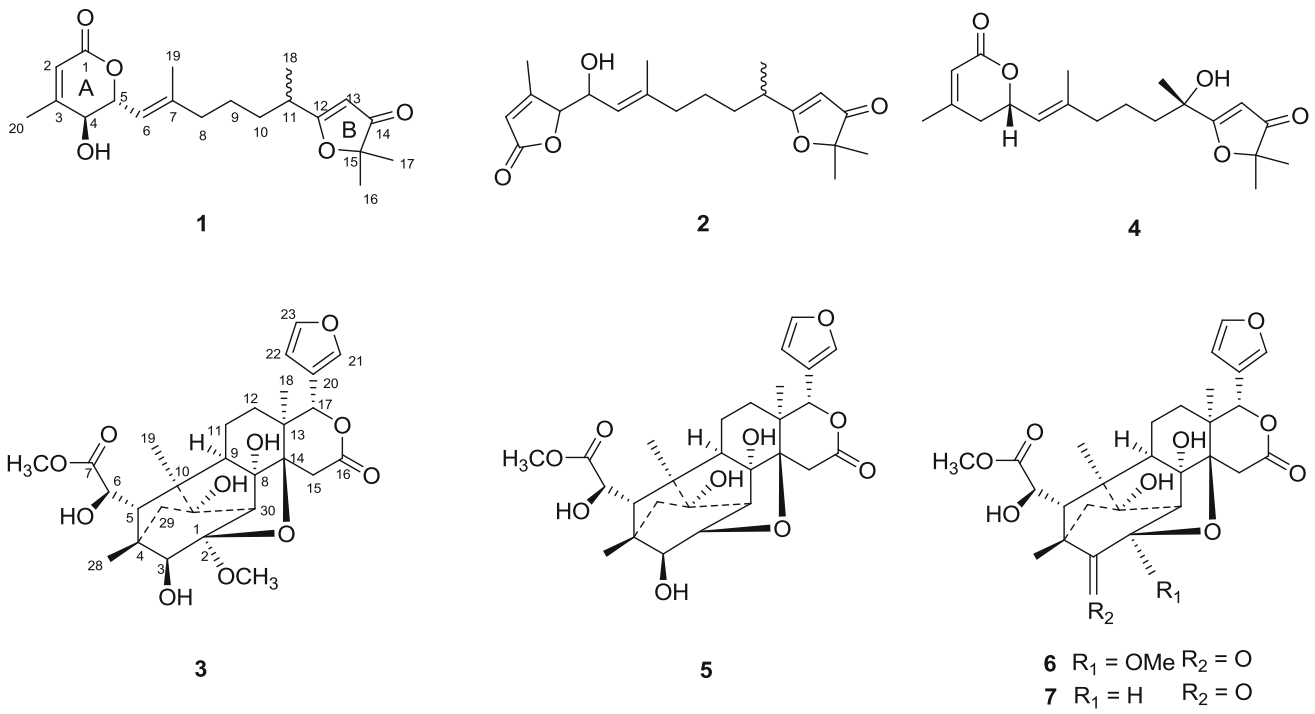

Fig. 1 The structure of compounds **1**–**7**

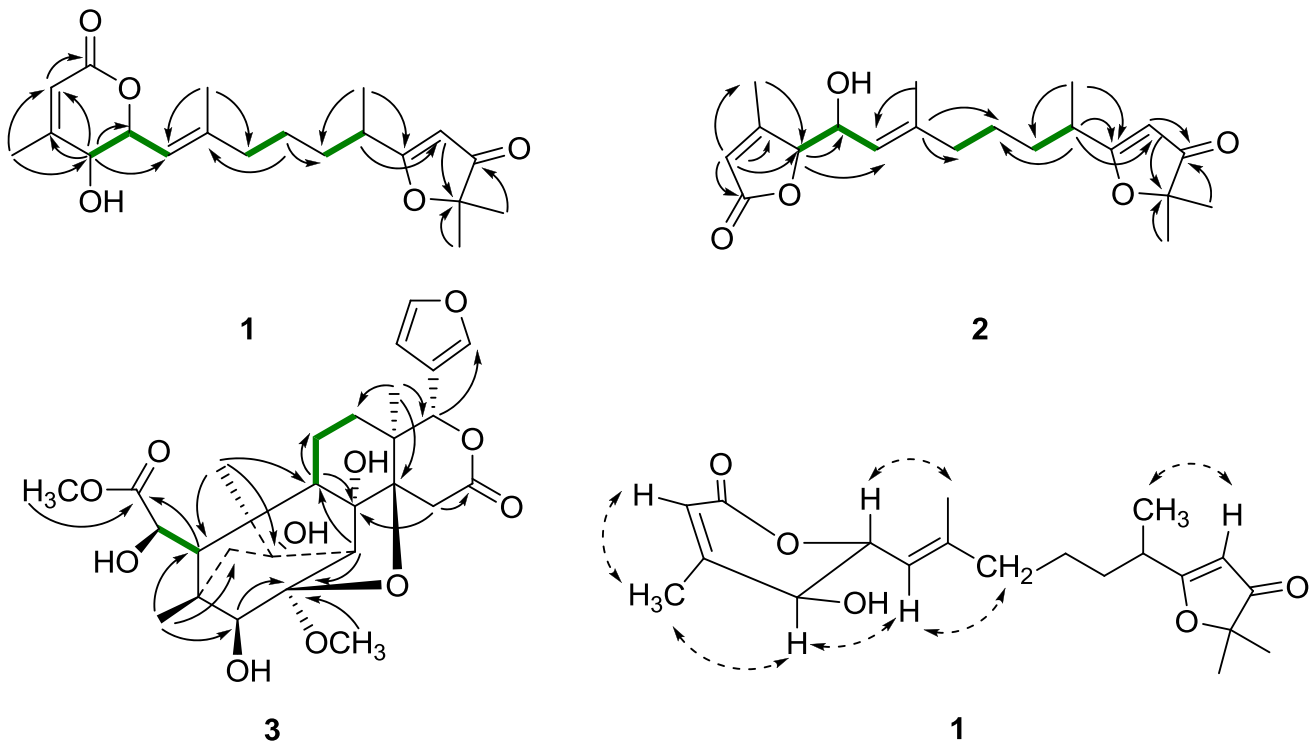

Fig. 2 ^{1}H-^{1}H COSY (▬) and key HMBC (⟶) correlations of **1**–**3** and key ROESY (⟷) correlations of **1**

showed the presence of three typical olefinic protons δ_H (5.80, s-like; 5.42, s; 5.26, dd, $J = 8.7, 1.2$ Hz), three methyl singlets at δ_H 1.35 (6H), 2.05 (3H) and two methyl doublets at δ_H (1.23, $J = 7.0$ Hz; 1.75, $J = 1.2$ Hz). The ^{13}C DEPT displayed five methyls, three methylenes, six methines, and six quaternary carbons (Table 1). 1D-NMR data of **1**

Table 1 ^{1}H and ^{13}C-NMR spectroscopic data of compounds **1** and **2** (δ in ppm)

Pos.	**1**[a]		**2**[b]	
	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C
1		166.3 s		176.3 s
2	5.80 (s-like)	117.1 d	5.83 (s)	118.9 d
3		162.6 s		170.7 s
4	4.04 (d, 7.2)	69.7 d	4.89 (overlap)	89.3 d
5	4.99 (dd, 8.7, 7.2)	80.5 d	4.70 (dd, 8.8, 3.1)	68.2 d
6	5.26 (dd, 8.7, 1.2)	121.9 d	5.20 (d, 8.8)	124.3 d
7		145.4 s		142.1 s
8	2.10 (m), 2.10 (m)	40.5 t	2.03 (m), 2.03 (m)	40.7 t
9	1.49 (m), 1.49 (m)	25.9 t	1.44 (m), 1.44 (m)	26.5 t
10	1.64 (m), 1.52 (m)	34.7 t	1.60 (m), 1.49 (m)	35.0 t
11	2.71 (m)	36.9 d	2.68 (m)	37.3 d
12		198.9 s		199.5 s
13	5.42 (s)	100.7 d	5.41 (s)	101.1 d
14		210.3 s		210.7 s
15		89.9 s		90.3 s
16	1.35 (s)	23.3 q	1.32 (s)	23.5 q
17	1.35 (s)	23.3 q	1.32 (s)	23.5 q
18	1.23 (d, 7.0)	18.3 q	1.20 (d, 6.9)	18.4 q
19	1.75 (d, 1.2)	17.1 q	1.68 (s)	17.0 q
20	2.05 (s)	19.9 q	2.13 (s)	15.0 q

[a] Recorded in CD_3OD and $CDCl_3$; ^{1}H and ^{13}C-DEPT Recorded at 600, 150 MHz

[b] Recorded in CD_3OD, ^{1}H and ^{13}C-DEPT Recorded at 500, 125 MHz

Table 2 ^{1}H and ^{13}C-NMR spectroscopic data of compound **3** (δ in ppm)

Pos.	δ_H (J in Hz)	δ_C	Pos.	δ_H (J in Hz)	δ_C
1		85.1 s	15	3.82 (d, 18.3), 4.20 (d, 18.3)	37.8 t
2		106.9 s	16		172.4 s
3	3.78 (d, 5.9)	84.7 d	17	6.26 (s)	81.7 d
4		43.7 s	18	1.39 (s).	16.3 q
5	3.50 (d, 8.4)	41.4 d	19	1.89 (s)	19.2 q
6	4.74 (d, 8.4)	72.3 d	20		122.7 s
7		176.7 s	21	7.55 (br.s)	142.1 d
8		87.9 s	22	6.54 (br.s)	111.5 d
9	2.96 (d, 9.2)	57.3 d	23	7.62 (br.s)	143.7 d
10		61.3 s	28	1.39 (s)	20.6 q
11	2.11 (m), 2.51 (m)	17.7 t	29	1.94 (d, 11.8), 2.39 (d, 11.8)	46.0 t
12	1.22 (m), 2.51 (m)	28.3 t	30	3.55 (s)	74.5 d
13		38.9 s	OMe-7	3.73 (s)	52.4 q
14		84.7 s	OMe-2	3.68 (s)	51.2 q

Recorded in C_5D_5N; ^{1}H and ^{13}C-DEPT Recorded at 600, 150 MHz

exhibited high resemblance with nemoralisin A isolated from *Aphanamixis grandifolia* (Meliaceae) [13], and they showed the same molecular formula and the similar skeletal structure (α,β-unsaturated ketone was connected with one α,β-unsaturated δ-lactone by an aliphatic chain). The only difference between **1** and nemoralisin A was that the hydroxyl group located at C-4 in **1** but at C-8 in nemoralisin A. And this was confirmed by HMBC correlations of H-4 (δ_H 4.04, d, J = 7.2 Hz) with C-2 (δ_C 117.1), C-3 (δ_C 162.6), C-5 (δ_C 80.5), C-6 (δ_C 121.9), and of Me-20 (δ_H 2.05) with C-4 (δ_C 69.7), and together with by the ^{1}H-^{1}H COSY cross-peaks between H-4 and H-5 (δ_H 4.99, d, J = 8.7, 7.2 Hz) (Fig. 2).

In the ROSEY spectrum, the strong correlation from H-6 (δ_H 5.26, dd, J = 8.7, 1.2 Hz) to H-8 (δ_H 2.10, m) inferred E-geometry of the Δ^6 double bond. This was confirmed by the upfield resonance of vinylic Me-19 group at δ_C 17.1 [14]. The ROESY correlations of H-4/H-6 and H-5/Me-19 (δ_H 1.75, d, J = 1.2 Hz) suggested that H-4 and H-5 was *trans* oriented. However, the absolute configuration was not determined on the basis of 1D and 2D NMR data. Accordingly, compound **1** was established and named as nemoralisin H.

Compound **2** was isolated as colorless oil. The molecular formula of $C_{20}H_{28}O_5$ was determined by the positive ion peak at *m/z* 348.1927 $[M]^+$ (calcd for 348.1937) in the HREIMS. Extensive analysis of the 1D-NMR spectroscopic data (Table 1) of **2** exhibited a close resemblance with nemoralisin [14]. However, downfield shifts of the lactone carbon (δ_C 176.3), the β-sp^2 carbon (δ_C 170.7), and the oxygenated methine (δ_C 89.3) were observed in the ^{13}C DEPT spectrum of **2**, which implied the α,β-unsaturated δ-lactone (ring A) in nemoralisin was replaced by an α,β-unsaturated γ-lactone in **2**. This deduction was confirmed by the observed HMBC correlations of H-4 (δ_H 4.89) with C-1 (δ_C 176.3), C-2 (δ_C 118.9), C-3 (δ_C 170.7), C-6 (δ_C 124.3), and of Me-20 (δ_H 2.13) with C-4 (δ_C 89.3) (Fig. 2). Meanwhile, on the basis of ^{1}H-^{1}H COSY correlations of H-4/H-5/H-6, a hydroxyl group at C-5 and Δ^6 double bond were established. The ROESY correlations of H-6 (δ_H 5.20, d, J = 8.8 Hz) with H-8 (δ_H 2.03, m) inferred E-geometry of the Δ^6 double bond. Therefore, the structure of **2** was determined and gave the name nemoralisin I.

Compound **3**, white amorphous powder, was found to possess the molecular formula of $C_{28}H_{36}O_{11}$ as inferred by HREIMS peak at *m/z* 548.2250 $[M]^+$ (calcd for 548.2258). The 1D NMR data (Table 2) of **3** indicated that it was a phragmalin-type limonoid and similar with khayseneganin E [15–17]. However, a methoxy in **3** replaced the hydroxyl group at C-2 in khayseneganin E, which was supported by

HMBC correlation between OMe (δ_H 3.68) and the ketal carbon (δ_C 106.9) (Fig. 2). The relative configuration of **3** was determined by analysis of the ROESY spectrum, in which correlations of H-5/H-6, H-5/H-12β, H-12β/H-17, and H-6/Me-28 suggested that these groups are all β-oriented. In addition, Me-19, Me-18, H-9, and H-3 were assigned as α-oriented on the basis of the ROESY correlations between Me-19/H-9 and between H_2-29/H-3, Me-18/H-3, suggesting that OH-3 is β-oriented. Consequently, the structure of **3** was determined as shown, named 2-methoxy khayseneganin E.

Four known constituents: nemoralisin C (**4**) [13], khayanolide B (**5**) [18], khayseneganin G (**6**) [17], and deacetylkhayanolide E (**7**) [19], were identified by the comparison of their spectroscopic data with those reported in the literature.

Compounds **1**–**7** were tested for in vitro inhibitory activities against HL-60, SMMC-7721, A549, MCF-7 and SW480 human tumor cell lines. All the tested samples showed no activities against the mentioned cell lines with $IC_{50} > 40$ μM. Much to our delight, Liu J. et al. [20] reported that aphadilactones A−D, which can be considered as the dimers of diterpenoid compounds (**1**, **2** and **4**) showed potent and selective inhibition against the diacylglycerol *o*-acyltransferase-1 (DGAT-1) enzyme, and are the strongest natural DGAT-1 inhibitors discovered to date. So emphasis can be laid on this class of compounds in our future search of potential DGAT-1 inhibitors.

3 Experiment Section

3.1 General Experimental Procedures

Optical rotations were obtained with a Jasco P-1020 polarimeter. UV (in MeOH) and IR (in $CHCl_3$) spectra were measured on Shimadzu UV-2401 PC spectrophotometer and Bruker Tensor-27 infrared spectrophotometer, respectively. ESIMS spectra were recorded on an API QSTAR Pulsar spectrometer. EIMS and HREIMS were performed on a Waters Autospec Premier P776. 1D and 2D NMR spectra were recorded on Bruker DRX-500 and Bruker Avance III-600 MHz spectrometers. Chemical shifts (δ) were expressed in *ppm* with reference to the TMS resonance. Semi-preparative HPLC studies were carried out on an Agilent 1100 liquid chromatograph with a Zorbax SB-C18 (9.4 mm × 25 cm) column. Column chromatography was performed using Silica gel [(200–300) mesh, Qingdao Marine Chemical, Inc, Qingdao, China]. Fractions were monitored by TLC and spots were visualized by heating the silica gel plates sprayed with 10 % H_2SO_4 in EtOH. Lichroprep RP-18 [(40–63) μm, Merck] and Sephadex LH-20 [(20–150) μm, Pharmacia] were also used for column chromatography.

3.2 Plant Material

The leaves and twigs of *S. mahagoni* were collected from Xishuangbanna, Yunnan Province, China. A voucher sample has been deposited in the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences.

3.3 Extraction and Isolation

The air-dried powdered leaves and twigs of *S. mahagoni* (10 kg) were extracted with 70 % aqueous acetone (each 30 L, 3 days) at room temperature three times to give a dark green residue (650 g), which was then partitioned between EtOAc and water to give the EtOAc-soluble fraction (120 g). The EtOAc extract was chromatographed by silica gel column eluted with $CHCl_3$–MeOH as a gradient (100:1, 50:1, 20:1, 5:1) to afford four fractions. The $CHCl_3$–MeOH (100:1) portion was evaporated to obtain a residue (20 g), which was subjected to silica gel chromatograph column with petroleum ether–EtOAc (10:1, 6:1, 3:1, 1:1) as elution, to give fractions (A, B, C, D). Fraction B was further subjected to RP-18 chromatograph column, eluting with MeOH–H_2O (50:50, 65:35, 80:20) to afford subfraction (E), which was then purified by semi-preparation HPLC with MeCN–H_2O (50:50) to give compound **3** (6 mg), **5** (12 mg), **6** (10 mg), **7** (15 mg). Fraction C was subjected to silica gel chromatograph column with petroleum ether–EtOAc (8:1, 5:1, 3:1, 1:1) as elution, to give fractions F, which was successively subjected to RP-18, Sephadex LH-20 and semi-preparation HPLC, compound **1** (1.5 mg), **2** (1.3 mg), and **4** (6 mg) were obtained.

3.4 Nemoralisin H (**1**)

Colorless oil; $[\alpha]_D^{25}$ − 0.48 (*c* 0.11, MeOH); UV (MeOH) λ_{max} (log ε) 202 (3.75), 261(3.37) nm; IR (KBr) ν_{max} 3434, 2976, 2930, 1761, 1639, 1585, 1457, 1383, 1270, 1177, 1088, and 1036 cm^{-1}, ^1H and ^{13}C-DEPT data see Table 1; EIMS *m/z* 371 $[M + Na]^+$; HREIMS *m/z* 348.1932 (calcd for $C_{20}H_{28}O_5$ $[M]^+$, 348.1937).

3.5 Nemoralisin I (**2**)

Colorless oil; $[\alpha]_D^{25}$ − 14.3 (*c* 0.18, MeOH); UV (MeOH) λ_{max} (log ε) 204 (3.87), 261(3.70) nm; IR (KBr) ν_{max} 3432, 2977, 2933, 1761, 1698, 1584, 1458, 1383, 1299, 1267,

1177, 1034 and 938 cm^{-1}, ^{1}H and ^{13}C-DEPT data see Table 1; ESIMS *m/z* 371 $[M + Na]^+$; HREIMS *m/z* 348.1927 (calcd for $C_{20}H_{28}O_5$ $[M]^+$, 348.1937).

3.6 2-Methoxy khayseneganin E (**3**)

White amorphous powder; $[\alpha]_D^{18} + 26.3$ (*c* 0.03, MeOH); UV (MeOH) λ_{max}(log ε) 209 (3.83) nm; IR (KBr) ν_{max} 3440, 2952, 1727, 1632, 1462, 1441, 1387, 1280, 1245, 1159, 1053, 1024, and 600 cm^{-1}, ESIMS *m/z* 571 $[M + Na]^+$; HREIMS *m/z* 548.2250 (calcd for $C_{28}H_{36}O_{11}$ $[M]^+$, 548.2258).

3.7 Cytotoxicity Assay

The cytotoxic activities of compounds **1**–**7** against HL-60, SMMC-7721, A549, MCF-7 and SW480 cell lines were determined by the MTT method [21].

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